Podophyllotoxin acetate blocks IR-induced invasion of non-small cell lung cancer cell, A549

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1. Introduction

γ- Ionizing radiation (IR) is extensively used in cancer therapy (1–3). But, some of previous studies showed that IR increases the invasiveness of cancer cells including glioma, hepatocellular carcinoma, pancreatic cancer cells. Moreover, some research result presented that local radiotherapy administered to primary tumors speeds their metastatic growth *in vivo* (4-6), thereby suggesting that besides its therapeutic effects, IR promotes the malignant behaviors of surviving cancer cells. Our findings demonstrate podophyllotoxin acetate (PA), one of new natural products, prevented side effects of IR such as invasion or metastasis promotion for improve the efficacy of radiotherapy.

2. Methods

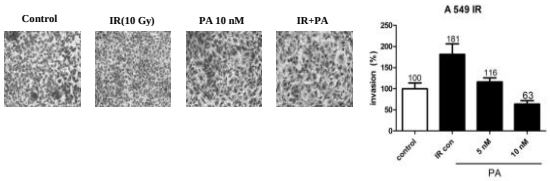
2.1 Cell culture and Radiation, PA treatment

Human A549 lung cancer cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum. Cells were maintained at 37℃ in 5% CO2. To irradiate the cells were plated on 60 mm dishes, incubated until 70–80% confluence was reached, and IR exposure (2 Gy; dose rate 0.6 Gy/min) using Gamma cell Cesium-137 unit. PA was treated after IR exposure 24h.

2.2 PA inhibited of IR-induced invasion and migration

Enhanced invasion and migration of A549 cells following γ-IR treatment was detected based on quantitative analysis using matrigel-coated transwells (Fig. 1).
Matrigel coated transwells (Corning NY, USA) were performed cell invasion and Collagen coated transwells (Corning NY, USA) were performed cell migration. Cells ($1x10^4$) in 200 ul of medium were seeded onto the upper chamber in transwells. Serum-free media supplemented with 0.1% bovine serum albumin in lower chamber. Transwells were incubated for 18h with 5% CO_2 at 37 ℃. Cell staining was performed with deep quick solution (Merck, Whitehouse Station, NJ,USA).

(A) invasion



(B) migration

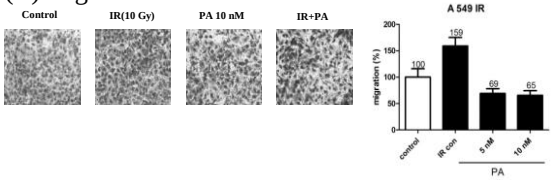
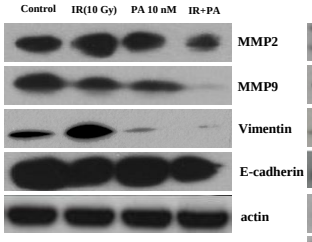


Fig. 1. IR induced increase of invasion and migration. PA decreased IR-induced invasion and migration in A549 cell.

2.3 PA regulated EMT and MAPKinase pathway

Intracellular signaling mechanism of γ-IR – induced invasion and migration of A549 cells following γ-IR treatment was detected based on western blotting as follows:
RIPA buffer [50 mM Tris, pH.8.0, 150 mM NaCl, 1% NP-40, 0.5% deoxycholic acid, and 0.1% sodium dodecyl sulfate (SDS)] containing protease and phosphatase inhibitor was used to dissolve harvested cell pellets for acquiring whole-cell protein lysates. Cell lysates were separated by 12% SDS -polyacrylamide gel electrophoresis (PAGE) and protein were transferred to nitrocellulose membranes. The membrane was incubated with blocking buffer (containing 5% skim milk in 0.1% PBS-T) at room temperature and then was washed in 0.1% PBS-T. After theses step, the blot was incubated with protein-specific primary antibody and then was incubated with labeled with HRP conjugated secondary antibody.

(A) EMT pathway



(B) MAPKinase pathway

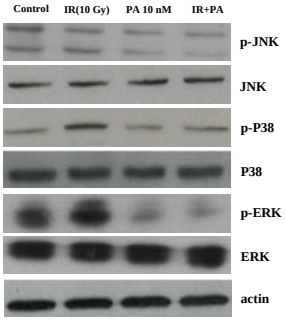


Fig. 2. PA suppressed EMT and MAPKiase pathway in IR-treated A549 cells.

3. Conclusions

Previous our report has shown that IR enhances the invasiveness of A549 cells (8). In this study, we demonstrated that PA inhibits IR-induced invasion and migration of A549 cells. We also observed that IR stimulates several intracellular pathway involving EMT and MAPKinses; EMT-associated events including an increase of vimentin levels and increased phosphorylation of p38 ERK, JNK in A549 cells. PA could decrease these activations of several intracellular signaling molecules. Therefore, PA might inhibit IR-induced invasion and migration via blocking EMT and MAPKiase pathway of A549 cells. Taken together, our results present that potential ability of PA improve efficacy of radiotherapy via suppression of invasion as well as induction of cancer cell death during radiotherapy.

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